S/PRTS

10/537248

JC17 Rec'd PCT/PTO 0 1 JUN 2005

1

Description

Method and device for transporting or binding-specific separation of electrically charged molecules

5

The invention relates to a method for transporting or binding-specific separation of electrically charged molecules in an aqueous solution, in particular during the operation of a DNA sensor with a redox cycling process between measuring

10   electrodes. The invention additionally relates to the associated devices.

The transporting of charged particles in an electric field (migration) plays an important part in numerous methods of

15   molecular biology. The migration velocity v of the charged particles in the liquid medium is in this case proportional to the field strength E and the ion charge Q and inversely proportional to the particle radius r and the viscosity η of the suspension. The following results for the velocity v:

$$v = QE/6\pi r\eta \qquad (1)$$

20

During electrophoresis, by way of example, biomolecules, i.e primarily proteins and DNA, which differ with regard to their size and/or charge are separated from one another. The presence of other mobile charged particles is to be avoided in certain

25   forms of electrophoretic separation (e.g. isoelectric focusing) since otherwise the charge transport is undertaken partly or wholly by these particles and not by the molecules to be separated. Therefore, amino acids that have their isoelectric point at the desired pH value are often used as a buffer. That

30   is to say that, at the pH value set, the buffer molecules themselves have no net charge and are therefore not subject to migration.

Electric fields are also used in the transporting of charged molecules, e.g. in order to increase or to decrease the concentration at a specific location. Particularly in the case of microsensors, e.g. for DNA analysis, it is possible to
5   increase the sensitivity if the DNA fragments (target molecules) to be detected are concentrated at the location of the capture molecules (sensor surface). The number of capture/target molecule bonds thus increases in accordance with the law of mass action. In any event, however, during such a
10   reaction not only are capture/target molecule pairs formed which match one another exactly but also those whose sequence do not correspond to one another exactly at some sites (mismatches).

15   Since the magnitude of the binding energy decreases with the number of non-corresponding bases, those bonds which have a specific number of mismatches can be separated again selectively by the application of appropriate forces (stringency treatment). As force, it is possible here for an
20   electric field to take effect which has an opposite polarity in contrast to the first process, the concentration of the molecules.

A prerequisite for transporting charged particles in the
25   electric field is a field gradient that has a strictly monotonic profile within the electrolyte or the transport path. That is to say that the field gradient must not change its sign and must not become zero. The application of an arbitrary voltage is not necessarily sufficient for this purpose for
30   aqueous systems. In the absence of a chemical reaction before the electrodes, the voltage drops across the electrochemical double layer and the field gradient between the electrodes becomes zero. However, if a reduction or oxidation reaction takes place at the electrodes, the double layer before the

electrodes is depolarized and the electric field has a strictly monotonic profile within the electrolyte. Ion transport in the aqueous electrolyte is the consequence.

A method that is frequently employed for generating such electric fields in aqueous systems is application of the decomposition voltage of water. In this case, oxygen is evolved at the anode and hydrogen at the cathode. In the experimental
5   implementation, care must be taken to ensure that the gases, and in particular their free radical precursors do not come into contact with the molecules to be examined, since the latter would otherwise be altered chemically. In macroscopic systems, this is done by separating the electrolyte spaces
10 directly before the electrodes from the electrolyte space between the electrodes, e.g. by means of diaphragms. This solution is problematic for microsensors since diaphragms are not practicable.

15 One possibility for electrophoresis in microsystems consists in introducing so-called permeation layers made of hydrophilic polymer before the electrodes, in respect of which reference is made to US 5 605 662 A. The mobility of reaction products of the electrolysis of water and the DNA to be transported is
20 severely inhibited in this layer, so that an intermixing virtually does not take place. The charge transport in the permeation layer is undertaken by smaller ions.

Although the known method is practicable, the introduction of
25 new polymer layers makes the production of the microsensor chip significantly more complicated and thus more expensive.

Therefore, it is an object of the invention to specify a suitable method for transporting the charged molecules by means
30 of an electric field, in the case of which no evolution of hydrogen or oxygen occurs at the electrode. In particular, with utilization of the electrophoresis method, the intention is to create a corresponding device that manages with standard materials and layers of chip production.

The object is achieved with regard to the method by means of the features alternatively of patent claim 1 or of patent claim

12. An associated device is specified in patent claim 15. The respective dependent claims relates to developments of the method and of the device.

5   In the case of the device according to the invention, a construction that is identical, in principle, can be used optionally to perform the method according to the invention in accordance with patent claim 1 or the method according to the invention in accordance with patent claim 12. In this case, it
10   is also advantageously possible to combine both methods with one another, for example cyclically.

In the application of the electrophoresis method, the invention makes use of the fact that, in addition to the electrolysis of
15   water, other reactions can also be used for generating the electric field in the analyte solution. The invention proposes a metal/metal ion complex, e.g. copper/copper-histidine complex, as a depolarizer before the electrodes. In the event of positive polarization of a copper-coated electrode for the
20   purpose of concentrating negatively charged ions, oxygen is not then evolved; instead, the copper goes into solution as ion. If a complexing agent for the metal, e.g. histidine for copper is present there, then the metal iron remains stably in solution. Since e.g. the copper-histidine complex is very stable, the
25   concentration of the free copper ions remains very small and virtually constant. An influence of the copper ions on the DNA hybridization is thereby avoided.

If the electrode is intended to be negatively polarized in
30   order e.g. to increase the selectivity of the capture/target molecule binding (stringency treatment), i.e. to remove non-specifically bound, non-complementary sample DNA from the capture DNA, the metal ions are reduced in the presence of a metal ion complex of a sufficiently noble metal, e.g. copper,

and are deposited in the process on the electrodes (in this case the measuring electrode). Evolution of hydrogen is thereby avoided. The complexing agent for the metal ion may,

under certain circumstances, also serve simultaneously as a buffer. Histidine is used for example as a buffer at pH = 7. The copper deposited on the measuring electrodes can be removed in a washing step by renewed application of negative potential.

5   A repulsion of the target molecules is prevented by using a washing solution with high ionic strength, so that only e.g. copper in the form of Cu^{2+} ions is removed, but the target DNA is not moved.

10   The advantage of an electrophoresis method based on metal/metal ion complex resides in the lower voltage required for generating the electric field. It is lower than the electrolysis voltage of water, so that the aggressive products of the electrolysis of water cannot arise. A separation of

15   electrolysis space and electrophoresis space thus becomes unnecessary. The generated field nevertheless suffices to transport the desired molecules in the analyte.

Copper is already used nowadays for interconnects and may be

20   used in the future as an electrode material for sensor applications or microsystems engineering applications such as micro-electrophoresis. In the production of such a microsystem it is therefore possible to have recourse to cost-effective standard methods of semiconductor technology.

25

Further details and advantages of the invention emerge from the following description of figures of exemplary embodiments with reference to the drawing in conjunction with the patent claims. In the figures:

30

Figure 1   shows a basic construction for carrying out the method according to the invention,

Figures 2 to 4   show cross sections of differently formed arrangements,

6

Figures 5a and 5b   show, in the case of arrangements in
                         accordance with figure 3, in method terms,
                         the enrichment of target molecules from low
                         to high concentration,

5   Figures 6a and 6b   show, in method terms, a situation in
                         accordance with figure 5b, in which,
                         however, non-specific, i.e. non-
                         complementary sample DNA are also present,
                         which are subjected to a so-called
10   stringency treatment,

     Figure 7   shows the electrode process in the case of
                         the invention's use of a sacrificial
                         electrode, and also of a complexing agent,

     Figures 8 to 10   show plan views of different measuring
15   electrode configurations,

     Figure 11   shows a measuring arrangement with
                         measuring positions arranged next to one
                         another, in cross section, and

     Figure 12   shows an array arrangement formed from
20   individual positions corresponding to
                         figure 8, in plan view.

The figures will be described together in part.

25   The basic construction of a general arrangement for carrying
     out biochemical measurements can be seen from figure 1. 1
     designates a planar substrate, e.g. made of silicon, on which a
     thin insulator layer 2, e.g. made of silicon oxide (SiO_2), is
     applied. Two measuring electrodes 20 and 30, which preferably
30   comprise noble metal, in particular gold, are situated on this
     arrangement. The entire measuring arrangement is in contact
     with an aqueous solution 15.

     The aqueous solution 15 contains negatively charged
35   macromolecules, this being illustrated by the bundle structure

in figure 1, and which are designated specifically by 200, 200'
further below in figure 5. The negatively charged molecules are
intended to be transported to the measuring electrodes 20, 30
and are also referred to hereinafter as target molecules. In
5   the case

of a DNA analysis, the target molecules of the DNA to be examined. By means of capture molecules that can be immobilized e.g. in a hydrogel layer 35, it is possible to attach the target DNA for the purpose of measurement in the vicinity of
5   the electrodes 20, 30.

In the aqueous solution 15 there is furthermore present a material which is resistant in the aqueous solution and more electronegative than the metal of the measuring electrodes. In
10   the most general case, the material is a metal/metal ion (Me/Me^+) combination, for example Cu/Cu^{2+}. This means that, in accordance with the predetermined potential conditions, either metallic copper Cu^o is dissolved with two electrons being released or copper(II) ions Cu^{2+} can be deposited with two
15   electrons being taken up, in which case the following holds true:

$$Cu^o \Leftrightarrow Cu^{++} + 2e \qquad\qquad (2)$$
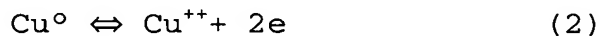
20   In the case of the arrangement in accordance with figure 5, in the case of a copper electrode as sacrificial anode 40, Cu^{2+} can go into solution as a result of a positive potential being applied. As a result, the negative target molecules 200 are moved there to the copper electrode 40 and accumulate in the
25   vicinity thereof and thus also in the region of the measuring electrodes 20, 30.

If, with the presence of Cu^{2+} ions in the aqueous solution, a suitable negative potential is applied to the measuring
30   electrodes 20, 30 in accordance with figure 6, both capture molecule/target DNA bonds break which have a reduced binding strength on account of incomplete complementarity. At the same time, copper(II) ions (Cu^{2+}) are reduced to form metallic copper (Cu^o) at the measuring electrodes in the process.

7a

The methodical processes in accordance with the alternatives demonstrated only in principle in figure 1 are illustrated with reference to figures 5a, 5b, on the one hand, and 6a, 6b on the other hand, and also figure 7. Specifically in figures 5a to
5   6b, a hydrogel layer 35 is in each case applied above the measuring electrodes

20 and 30, which have sensor surfaces 21 and 31, said hydrogel layer enclosing capture molecules 100 for target molecules 200 situated outside the hydrogel 35. What is essential in this case is that the capture molecules 100 capture and bind the
5   target molecules 200 and thus supply them for analysis at the sensor surface 21 and 31, respectively. With regard to this methodology, reference is made for example to applicant's earlier application PCT/DE 02/01982.

10   The capture molecules 100 may be for example specific thiol-modified oligonucleotides. Target molecules 200 that are intended to be bound by the capture molecules 100 are the DNAs to be analyzed.

15   In general, a known measuring arrangement exhibits a state in accordance with figure 5a, in the case of which the target DNA is present only in low concentration above the capture DNA. It is difficult in this case to attain reliable measurement results. In the case of an arrangement in accordance with
20   figure 5b, by contrast, the target DNA is present in high concentration above the capture DNA, this being achieved by means of a DNA enrichment. Good measurement results can be obtained in this state.

25   In accordance with figure 6a, in addition to the complementary target DNA 200, incompletely complementary DNA fragments 200' also bind to the capture DNA. By means of a stringency treatment, non-specifically bound DNA can be selectively removed by applying respectively suitable potentials to the
30   electrodes. The non-specifically bound DNA is then repelled on account of its weaker binding forces.

It can be seen from figure 1 and also subfigures 5a and 5b that a desired enrichment of the target DNA is achieved by applying
35   specific potentials to the auxiliary electrode 40. In detail,

for this purpose an auxiliary electrode 40 made of base metal, for example copper, is chosen and a positive potential is applied to the auxiliary electrode 40. If

the entire arrangement is situated in an aqueous solution, Cu^{2+} ions go into solution. As a result, a field gradient arises and the negatively charged DNA molecules are attracted.

5   The latter process is essentially illustrated by figure 7. In particular, it can be seen here that the copper ion brought into solution is complexed, for which purpose histidine molecules 70 are used.

10   It can be seen from figure 1 and also subfigures 6a and 6b that a desired selection of the DNA is achieved by applying specific potentials to the measuring electrodes 20, 30 and auxiliary electrodes 40, 45. In detail, the measuring electrodes are polarized negatively and the auxiliary electrodes positively.
15   If the entire arrangement is situated in an aqueous solution containing copper(II) ions (Cu^{2+}), the latter are reduced to metallic copper (Cu^{0}) on the measuring electrodes 20, 30. As a result, a field gradient arises and the negatively charged, incompletely complementary DNA is repelled.

20

The two alternatives may proceed separately or else in combination. Target molecules are firstly enriched and then selected. However, it is also possible to perform only a selection.

25

Figures 2 to 4 illustrate different variants of sensor arrangements. In figure 2, the measuring electrodes 20, 30 formed from gold have free gold sensor areas 21, 31, to which the capture DNA 100 is bound. As an alternative, a hydrogel 35
30   containing capture DNA 100 is present in figure 3. Figure 4 specifically illustrates an arrangement in which, besides the actual measuring electrodes 20 and 30, a free reaction area 50 made of gold is furthermore present, to which the capture DNA 100 is bound in a dense arrangement. This has the advantage of
35   a high density of

capture DNA. However, in the production of the reaction area
50, it is necessary firstly to cover the measuring electrodes
20, 30 with copper or the like in order to prevent an
attachment of the catcher DNA 100 there. Copper layers 22 and
5   32, respectively, are present for this purpose in figure 4. In
all of the arrangements in accordance with figures 2 to 4 the
sacrificial electrode 40 is in each case arranged in the
vicinity of the measuring electrodes 20 and 30 in order, as a
result of copper going into solution, to build up the field
10   gradient and thus to effect the enrichment of the target DNA
200 in the vicinity of the measuring electrodes 20 and 30. The
measurement accuracy can thus be considerably improved as a
result.

15   Figures 8 to 10 illustrate the different variants of measuring
sensor in accordance with figures 2 to 4 in plan view.
Specifically in figure 8, a measuring sensor 80 is present
which comprises two comb electrodes 82 and 83 with intermeshing
electrode fingers, a single sacrificial electrode 84 being
20   arranged annularly around the comb electrodes.

A corresponding arrangement emerges from figure 9, here the
region of the comb electrodes being covered with the hydrogel
layer 85. A hydrogel layer of this type may be situated over
25   the entire measuring arrangement. Specifically in figure 10,
reaction areas 86 for the attachment of catcher molecules are
additionally present as well.

From the individual sensors in accordance with figures 8 to 10
30   it is possible to design arrays having n rows and m columns.
Figures 11 and 12 illustrate a complete arrangement having a
multiplicity of measuring sensors 80, 80', … which constitute
the n·m array. In this case, it is possible in principle to
construct the array with individual positions corresponding to
35   one of figures 8 to 10, in the case of which each individual

position has an annular copper sacrificial anode 84. In this case the auxiliary electrode 185 is arranged as a further ring around the entire n·m arrangement with the individual positions.

In accordance with figure 11, the complete arrangement 180 is situated in a container, e.g. a through-flow channel 150, with a cover 120, an inflow 121 and an outflow 122.